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PATENT ABSTRACTS OF JAPAN

(11)Publication number : 07-206838

(43)Date of publication of application : 08.08.1995

(51)Int.Cl.

C07D307/32

A61K 31/34

A61K 31/34

A61K 31/34

A61K 31/34

(21)Application number : 06-002729

(71)Applicant : IMMUNO JAPAN:KK

(22)Date of filing : 14.01.1994

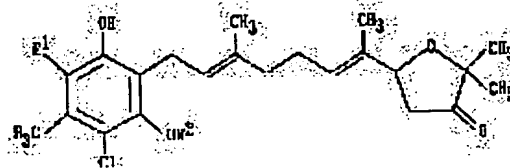
(72)Inventor : HOSOKAWA TOMOYOSHI

(54) NEW ASCOFURANONE DERIVATIVE AND BLOOD LIPID-LOWERING AGENT, HYPOGLYCEMIC AGENT, GLYCATION-INHIBITING AGENT AND ANTIOXIDIZING AGENT CONTAINING THE SAME AS ACTIVE INGREDIENT

(57)Abstract:

PURPOSE: To provide a new derivative high in water solubility, capable of being orally administered, low in acute toxicity, having excellent hypoglycemic, lipid- lowering, Maillard reaction-inhibiting, and antioxidizing actions, and useful as a medicine for preventing and treating diabete and arterial sclerosis.

CONSTITUTION: The derivative (salt) of the formula [R1 is -CHO, -COOH; R2 is H, -CH₂COOH, -CH₂COOR3 (R3 is 1-6C lower alkyl); but a case wherein R1 is -CHO and R2 is H or -CH₂COOR3 is excluded]. This derivative is obtained e.g. by reacting ascofuranone sodium salt with methyl bromoacetate in a dimethylformamide solution, adding hydrochloric acid to the reaction product, extracting the reaction mixture with chloroform, dissolving the extracted 4-O- methoxycarbonylmethyl ascofuranone in methanol, reacting the solution with a sodium hydroxide aqueous solution, adjusting the pH of the solution to 3 with hydrochloric acid, and subsequently extracting the solution with ethyl acetate.



LEGAL STATUS

[Date of request for examination] 13.07.2000

[Date of sending the examiner's decision of rejection] 18.03.2003

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

[Date of final disposal for application]

[Patent number]

[Date of registration]

[Number of appeal against examiner's decision of rejection]

[Date of requesting appeal against examiner's decision of rejection]

[Date of extinction of right]

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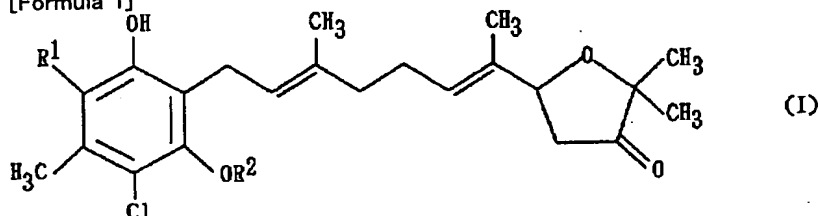
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CLAIMS

[Claim(s)]

[Claim 1] A general formula (I)

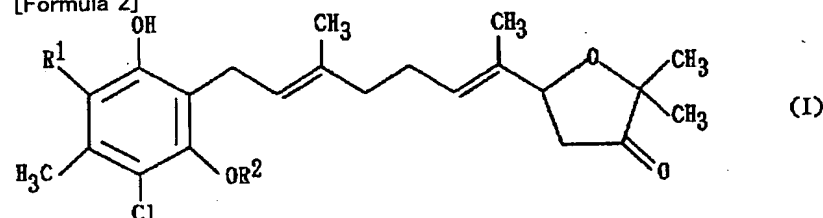
[Formula 1]



(As for the inside R1 of a formula, -CHO or -COOH is expressed and R2 expresses a hydrogen atom, -CH2COOH, or formula-CH2COOR3 (R3 expresses the low-grade alkyl group of a carbon number 1-6 among a formula).) R1 [however,] -CHO — and the case where R2 is a hydrogen atom or -CH2COOR3 (R3 expresses the low-grade alkyl group of a carbon number 1-6) — removing — ASUKO hula non shown — a derivative or its salt which can be permitted in pharmaceuticals.

[Claim 2] A general formula (I)

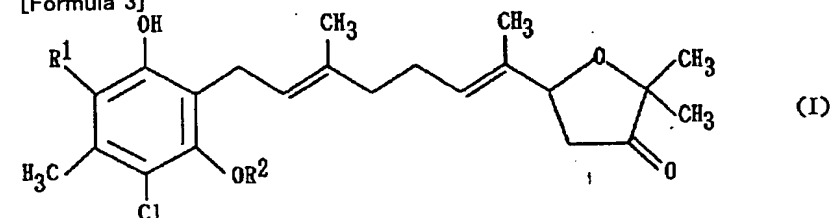
[Formula 2]



(As for the inside R1 of a formula, -CHO or -COOH is expressed and R2 expresses a hydrogen atom, -CH2COOH, or formula-CH2COOR3 (R3 expresses the low-grade alkyl group of a carbon number 1-6 among a formula).) R1 [however,] -CHO — and the case where R2 is a hydrogen atom or -CH2COOR3 (R3 expresses the low-grade alkyl group of a carbon number 1-6) — removing — ASUKO hula non shown — the lipid low laxative in blood which contains a kind as an active principle at least among a derivative and its salt which can be permitted in pharmaceuticals.

[Claim 3] A general formula (I)

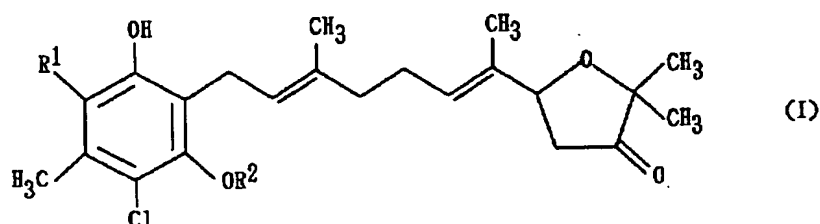
[Formula 3]



(As for the inside R1 of a formula, -CHO or -COOH is expressed and R2 expresses a hydrogen atom, -CH2COOH, or -CH2COOR3 (R3 expresses a carbon number 1-6 low-grade alkyl group among a formula).) R1 [however,] -CHO — and the case where R2 is a hydrogen atom or -CH2COOR3 (R3 expresses the low-grade alkyl group of a carbon number 1-6) — removing — ASUKO hula non shown — the blood sugar low laxative which contains a kind as an active principle at least among a derivative and its salt which can be permitted in pharmaceuticals.

[Claim 4] A general formula (I)

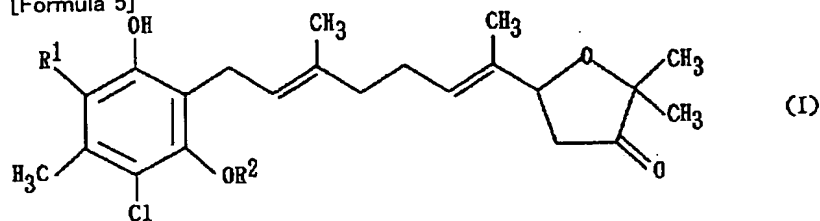
[Formula 4]



(As for the inside R^1 of a formula, $-CHO$ or $-COOH$ is expressed and R^2 expresses a hydrogen atom, $-CH_2COOH$, or formula- CH_2COOR^3 (R^3 expresses the low-grade alkyl group of a carbon number 1-6 among a formula).) R^1 [however,] $-CHO$ — and the case where R^2 is a hydrogen atom or $-CH_2COOR^3$ (R^3 expresses the low-grade alkyl group of a carbon number 1-6) — removing — ASUKO hula non shown — the GURIKEISHON inhibitor which contains a kind as an active principle at least among a derivative and its salt which can be permitted in pharmaceuticals.

[Claim 5] A general formula (I)

[Formula 5]



(As for the inside R^1 of a formula, $-CHO$ or $-COOH$ is expressed and R^2 expresses a hydrogen atom, $-CH_2COOH$, or formula- CH_2COOR^3 (R^3 expresses the low-grade alkyl group of a carbon number 1-6 among a formula).) R^1 [however,] $-CHO$ — and the case where R^2 is a hydrogen atom or $-CH_2COOR^3$ (R^3 expresses the low-grade alkyl group of a carbon number 1-6) — removing — ASUKO hula non shown — the anti-oxidant which contains a kind as an active principle at least among a derivative or its salt which can be permitted in pharmaceuticals.

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DETAILED DESCRIPTION

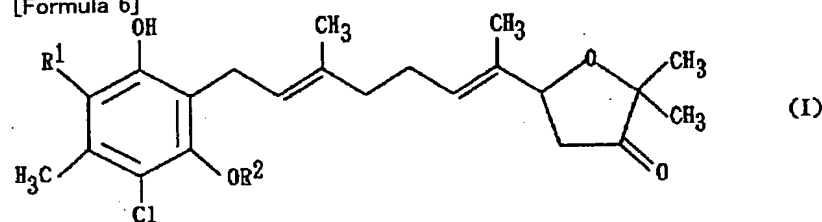
[Detailed Description of the Invention]

[0001]

[Industrial Application] This invention relates to the lipid low laxative in blood which makes a kind an active principle at least among these compounds at the new ASUKO hula non derivative shown by the following general formula (I), or its salt list which can be permitted in pharmaceuticals, a blood sugar low laxative, a GURIKEISHON inhibitor, and an anti-oxidant.

[0002]

[Formula 6]



(As for the inside R1 of a formula, -CHO or -COOH is expressed and R2 expresses a hydrogen atom, -CH₂COOH, or formula-CH₂COOR₃ (R₃ expresses the low-grade alkyl group of a carbon number 1-6 among a formula).) However, R1 is -CHO and the case where R2 is a hydrogen atom or -CH₂COOR₃ (R₃ expresses the low-grade alkyl group of a carbon number 1-6) is removed.

[0003]

[Description of the Prior Art] The ASUKO hula non R1 of the above-mentioned general formula (I) is shown by CHO, and R2 is indicated to be by H is Mold Ascochyta by this invention person. visiae Isolation is carried out from the inside of the fungus body of Libert (refer to JP,48-91278,A), and the antitumor activity is already reported (refer to JP,63-61929,B).

[0004] The ASUKO hula non derivative whose R2 R1 in the above-mentioned general formula is an acyl group in -CHO is indicated by the British patent No. 1,498,334 specification, and it is indicated that the derivative concerned has a blood-pressure descent operation.

[0005] The ASUKO hula non derivative whose R2 R1 in the above-mentioned general formula is OH in a hydrogen atom is indicated by JP,4-28705,B, and it is taught to it that it is useful as intermediate field for compounding ASUKO hula non.

[0006]

[Problem(s) to be Solved by the Invention] this invention person reported having the object for blood sugar low lean crops excellent in ASUKO hula non and some ASUKO hula non derivatives, as a result of repeating research wholeheartedly about the new usefulness as drugs of ASUKO hula non (Japanese Patent Application No. No. 18904 [five to])). however — these — a compound — (— one —) — water solubility — scarce — taking orally — like — prescribing a medicine for the patient — if — blood drug concentration — going up — being hard — (— two —) — taking orally — like — prescribing a medicine for the patient — if — an intestinal tract — inside — setting — esterase — easy — an ester bond — decomposing — (— three —) — usual — an oxidizing agent — receiving — very much — unstable — organic chemistry — like — a method — **** — one — place — an aldehyde group

[0007]

[Means for Solving the Problem] By carrying out alkali treatment of whether R2 carries out esterase processing of the compound shown by -CH₂COOR₃ beforehand, a compound of -CH₂COOH was obtained easily and an effect which was excellent in a blood sugar fall, a lipid fall, and Maillard reaction inhibitory action compared with the existing material was accepted. moreover, ASUKO hula non — a list — a grinding object of the derivative (1) animal liver — fixed time amount and a group which agitates or is known as (2) oxidative bacteria — if it is made to oxidize by bacteria, for example, a Pseudomonas group, or Acetobacter group, a CHO radical of the 1st place is convertible into a COOH radical. Thus, water solubility increases a compound with which the 1st obtained place oxidized to COOH, by internal use, blood drug concentration rises and bioavailability (bioavailability) increases it. Moreover, acute toxicity by internal use fell to 1/2-1/4.

[0008] Each purpose compound of this invention obtained by the above method has a blood sugar fall excellent in a new molecular entity, a lipid fall, Maillard reaction inhibition, and an antioxidation operation, and found out that it was

useful as drugs.

[0009] This invention was made based on such knowledge, and provides an ASUKO hula non derivative shown by the above-mentioned general formula (I) and its salt permitted in pharmaceuticals, and a list with a lipid low laxative in blood which contains a kind as an active principle at least among such compounds, a blood sugar low laxative, a GURIKEISHON inhibitor, and an anti-oxidant.

[0010] In various diseases, such as kidney disease, a digestive system disease, a circulatory system disease, and skin disease, an anti-oxidant of this invention is widely applicable in order to prevent oxidation.

[0011] When it is injection although each medicinal dosage concerning this invention changes with a class of symptoms, and symptoms for example, in ten to 200 mg per adult day, and internal use, in the case of 60 to 5000 mg, and a suppository, it can attain the purpose by 100 to 2000 mg. Although a compound of this invention may be used independently, it is desirable to manufacture medicine as dosage forms which usually neutralize with alkali, dissolve in water, or are mixed as suspension, an excipient, or other adjuvants, and fit parenteral administration and internal use. As desirable pharmaceutical preparation, injections, powder material, a granule, a tablet, a sugar-coated tablet, a round tablet, a capsule, a suppository, etc. are raised, for example. These pharmaceutical preparation is manufactured by conventional method, using a lactose, sucrose, various starch, grape sugar, a cellulose, methyl cellulose, a carboxymethyl cellulose, magnesium stearate, a lauryl sulfuric acid, talc, vegetable oil, lecithin, two or more sorts of such mixture, etc. as an excipient or an adjuvant.

[0012] A typical compound is shown below among compounds of this invention shown by general formula (I).

[0013]

A compound number R1 R2 R3 1 CHO CH₂COOH - 2 COOH CH₂COOR3 CH₃ 3 COOH CH₂COOH - 4 COOH H R2 by COOH in addition to the Still More Nearly Above-mentioned Compound - As a Compound of CH₂COOR3 [R1] R3 can mention compounds, such as C₂H₅, n-C₃H₇, i-C₃H₇, n-C₄H₉, s-C₄H₉, i-C₄H₉, t-C₄H₉, n-C₅H₁₁, i-C₅H₁₁, and n-C₆H₁₃.

[0014] Although this invention is explained at details based on an example below, these do not limit this invention at all.

[0015]

[Example 1] ASUKO hula non 10.08g was dissolved in dimethylformamide 120ml, and 0.8g of sodium hydride was added little by little to this 60%. Bromoacetic acid methyl ester 6g was added to a solution of obtained sodium salt. After carrying out overnight neglect, 0.08g [of sodium hydride] and bromoacetic acid methyl ester 0.6g was added to a room temperature 60 more%. Vacuum concentration was carried out after carrying out overnight neglect. 400ml [of hydrochloric acids] and chloroform 400ml was added to oily matter which remained 1%, and it agitated in a separating funnel, and a chloroform layer was isolated preparatively and concentration hardening by drying was carried out. When a methanol is added to oily matter which remained and overnight neglect is carried out, it is 4-O-methoxycarbonylmethyl. 7.6g of crystals of ascofuranone (AF-3913) deposited.

[0016] Obtained 4-O-methoxycarbonylmethyl ascofuranone 2g was dissolved in a 80ml methanol, and it was made to react at a room temperature in addition to a solution mixed with 30ml of 1-N sodium hydroxides 130ml of water. With after [30 minutes] ethyl ether, cooling, after washing, it was referred to as pH3 with 2N-hydrochloric acid, and extracted 3 times with ethyl acetate. When concentration hardening by drying of the ethyl-acetate layer is carried out after dehydration with anhydrous sodium sulfate after washing with saturation brine, it is 4-O-carboxymethyl. 1.4g of crystals of ascofuranone (a compound of the compound number 1, AF-M) was obtained.

[0017] NMR analytical data of a compound (AF-M) of the compound number 1 are shown below.

[0018] NMR delta(CDCl₃): — 12.51 (1H, s) and 10.27 (1H, s) — 5.51 (1H, t, J= 7.0Hz) 5.08 (1H, t, J= 6.2Hz), 4.65 (1H, dJ=15.8Hz), 4.64 (1H, m), 4.59 (1H, d, J= 15.8Hz), 3.46 (2H, d J=7.0Hz) 2.64 (3H, s), 2.49 (1H, dd, J= 18.3Hz, 6.6Hz) 2.44 (1H, dd, J= 18.3Hz, 10.1Hz), 2.18 (2H, m), 2.05 (2H, t J=7.0), 1.77 (3H, s), 1.62 (3H, s), 1.31 (3H, s), 1.25 (3H, s)

[0019]

[Example 2] Pseudomonas which carried out overnight culture at 28 degrees C 2% at a grape sugar addition bouillon culture medium at 2% grape sugar, 0.1% yeast extract, 0.2% peptone, 0.1% ammonium chloride, 0.05% phosphoric-acid-1-potash, 0.02% magnesium sulfate, and 10l. of culture culture media which consist of a calcium carbonate 0.5% ovalis 200ml was inoculated. Aeration spinner culture of the culture was carried out at temperature of 28 degrees C. ascofuranone dissolved in Tween80 15 hours after 20g was added and culture was continued further. Culture was terminated two days after, a sulfuric acid was added to culture filtrate obtained by dissociating with a centrifugal separator, and it adjusted to pH3. Next, 5l. of ethyl acetate was added and the churning extract was carried out. Vacuum concentration was carried out, after separating an ethyl-acetate layer and dehydrating with anhydrous sodium sulfate. Material of the compound number 4 was obtained by carrying out concentration hardening by drying of the fraction which performed a benzene silica gel column chromatography and was eluted in benzene-acetone (3:1) mixture in obtained oily matter.

[0020] NMR analytical data of a compound of the compound number 4 are shown below.

[0021] NMR delta(CDCl₃): — 9.66 (1H, s) and 5.43 (1H, brt) — 5.13 (1H, brt) 4.46 (1H, t J=8.3Hz), 3.30 (2H, d J=6.4Hz), 2.53 (3H, s), 2.36 (2H, m), 1.90-2.15 (4H, m), 1.74 (3H, s), 1.54 (3H, s), 1.11 (3H, s), 1.14 (3H, s)

[0022]

[Example 3] It is ASUKO hula (non AF) 4-O-methoxy carbonylmethyl to a ddY mouse of one groups [eight]. ASUKO hula non (AF-3913), 4-O-carboxymethyl ASUKO hula non (AF-M) was administered orally for 8mg [/kg] seven days, and an effect given to a blood cholesterol level and neutral fat in blood was examined. A result was shown in a table

1.

[0023]

[A table 1]

マウスにおける血中コレステロール、中性脂肪値

	n	コレステロール (mg/dl)	中性脂肪 (mg/dl)
対照群	8	116±10	199±13
AF	8	113±9 ns	159±10 -20% *
AF-3913	8	98±8 -16% *	146±12 -27% **
AF-M	8	95±8 -18% **	138±11 -31% **

平均±標準誤差 * p < 0.05 ** p < 0.01

Although a blood cholesterol level of an AF-M administration group and a neutral fat value fell intentionally compared with a control group, the decreasing rate was large compared with other two specimens.

[0024]

[Example 4] A glucose of 200mM(s) is added to 25mg [/ml] cow serum albumin, and it is the derivative (hydrolysate FUROSHIN of a keto amine which added at 40, 200, and a 1000 or 5000microg [/ml] rate, cultivated AF-3913.AF-M for 37 degrees C and 14 days, and generated it was measured with high performance chromatography.) to an ASUKO hula non (AF) list. A result was shown in a table 2.

[0025]

[A table 2]

フロシン生成に対する効果

阻害率 (%)				
μM	5000	1000	200	40
AF	22	20	5	0
AF-3913	23	19	6	0
AF-M	35	27	10	0

As compared with a case of two specimens of others [addition / AF-M], a rate of FUROSHIN generation inhibition showed a high price.

[0026]

[Example 5] 1000, 500, and pentosidine that did 100 or 20microM addition of, cultivated 37 degrees C for eight days, and was generated were measured for the derivative (AF-3913.AF-M) with high performance chromatography to reaction mixture containing a ribose of 10mM(s), an arginine, and a lysine at an ASUKO hula non (AF) list. A result was shown in a table 3.

[0027]

[A table 3]

ペントシジン生成に対する効果

阻害率 (%)				
μM	1000	500	100	20
AF	100	85	60	25
AF-3913	100	88	65	28
AF-M	100	95	77	38

As compared with a case of two specimens of others [addition / AF-M], a rate of pentosidine generation inhibition showed a high price.

[0028]

[Example 6] Ascorbic-acid 100microM and 25micro of ferric chlorides M were added to a rat liver mitochondrion, and 37 degrees C of effects of AF-3913 and AF-M over peroxy lipid cultivated and generated were examined for 1 hour. A result was shown in a table 4.

[0029]

[A table 4]

過酸化脂質生成に対する効果

	$\mu\text{g}/\text{ml}$	過酸化脂質	ng/ml
対照		62.1 \pm 1.1	
AF-3931	1000	9.6 \pm 0.6	-80.0% **
	100	47.2 \pm 1.8	-9.2% *
	10	55.1 \pm 2.4	ns
AF-M	1000	13.4 \pm 1.1	-74.8% **
	100	36.7 \pm 2.3	-30.9% **
	10	45.8 \pm 0.8	-13.8% *

平均 \pm 標準誤差 * $p < 0.05$ ** < 0.01

Although both compounds controlled generation of peroxy lipid, AF-M was more effective at low concentration as compared with AF-3913.

[0030]

[Effect of the Invention] Since the new ASUKO hula non derivative shown by the above-mentioned formula (I) or its salt which can be permitted in pharmaceuticals shows the outstanding hypolipidemic action in blood and the outstanding object for blood sugar low lean crops, GURIKEISHON inhibitory action, and an antioxidation operation, it is very useful as prevention of diabetes mellitus, arteriosclerosis, etc., and a remedy.

[Translation done.]

(19) 日本国特許庁 (J P)

(12) 公開特許公報 (A)

(11) 特許出願公開番号

特開平7-206838

(43) 公開日 平成7年(1995)8月8日

(51) IntCl.⁴
C 0 7 D 307/32
A 6 1 K 31/34

識別記号 庁内整理番号
A B X
A D N
A D P

F I

技術表示箇所

C 0 7 D 307/ 32

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審査請求 未請求 請求項の数 5 O L (全 6 頁) 最終頁に続く

(21) 出願番号 特願平6-2729

(22) 出願日 平成6年(1994)1月14日

(71) 出願人 391039391

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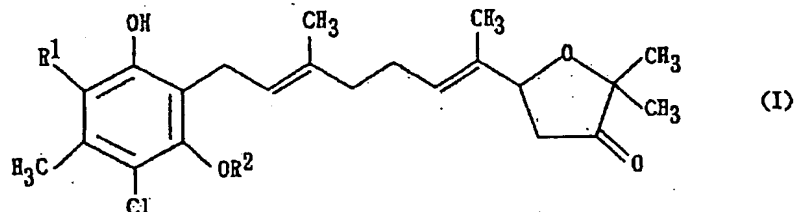
(74) 代理人 弁理士 湯浅 恭三 (外6名)

(54) 【発明の名称】 新規なアスコフラノン誘導体並びにそれらを有効成分とする血中脂質低下剤、血糖低下剤、グリケイション阻害剤及び抗酸化剤

(57) 【要約】

【構成】一般式 (I)

* 【化1】



(式中R¹は-CHO又は-COOHを表し、R²は水素原子、-CH₂COOH又は式-CH₂COOR³ (式中、R³は炭素数1-6の低級アルキル基を表す)を表す。但し、R¹が-CHOでかつR²が水素原子又は-CH₂COOR³ (R³は炭素数1-6の低級アルキル基を表す)である場合を除く)で示されるアスコフラノン誘導体

※導体又はその薬剤学的に許容し得る塩。

【効果】 上記式 (I) で示される化合物及びその薬剤学的に許容し得る塩は優れた血中脂質低下作用、血糖低下作用、グリケイション阻害作用及び抗酸化作用を示すので、糖尿病、動脈硬化症等の予防、治療薬として極めて有用である。

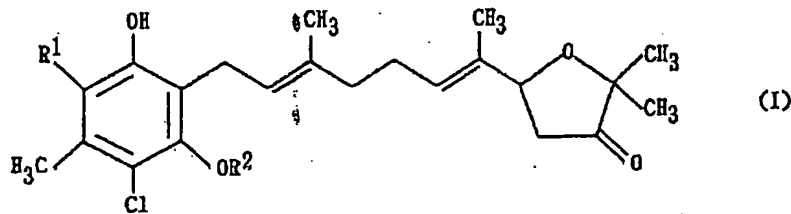
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【特許請求の範囲】

【請求項1】一般式 (I)

* 【化1】

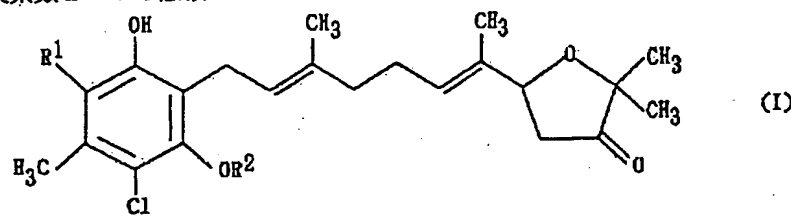


(式中R¹は-CHO又は-COOHを表し、R²は水素原子、-CH₂COOH又は式-CH₂COOR³ (式中、R³は炭素数1-6の低級アルキル基を表す)を表す。但し、R¹が-CHOでかつR²が水素原子又は-C

※表す)である場合を除く)で示されるアスコフラノン誘導体又はその薬剤学的に許容し得る塩。

【請求項2】一般式 (I)

【化2】

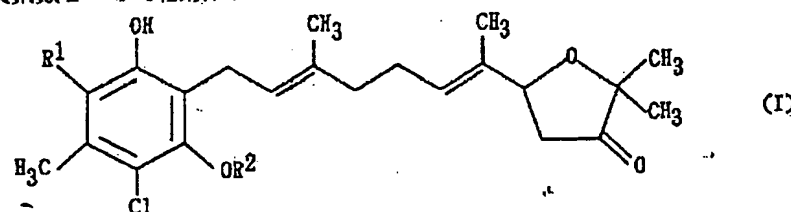


(式中R¹は-CHO又は-COOHを表し、R²は水素原子、-CH₂COOH又は式-CH₂COOR³ (式中、R³は炭素数1-6の低級アルキル基を表す)を表す。但し、R¹が-CHOでかつR²が水素原子又は-C

20★表す)である場合を除く)で示されるアスコフラノン誘導体及びその薬剤学的に許容し得る塩のうち少なくとも一種を有効成分として含有する血中脂質低下剤。

【請求項3】一般式 (I)

【化3】

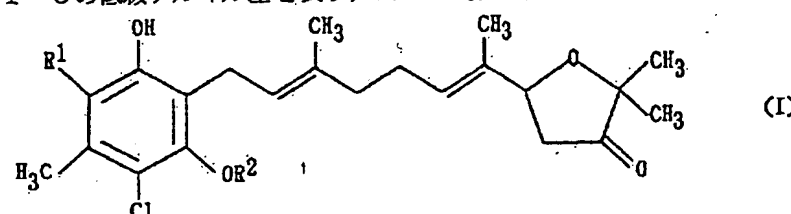


(式中R¹は-CHO又は-COOHを表し、R²は水素原子、-CH₂COOH又は-CH₂COOR³ (式中、R³は炭素数1-6低級アルキル基を表す)を表す。但し、R¹が-CHOでかつR²が水素原子又は-CH₂C

☆である場合を除く)で示されるアスコフラノン誘導体及びその薬剤学的に許容し得る塩のうち少なくとも一種を有効成分として含有する血糖低下剤。

【請求項4】一般式 (I)

【化4】

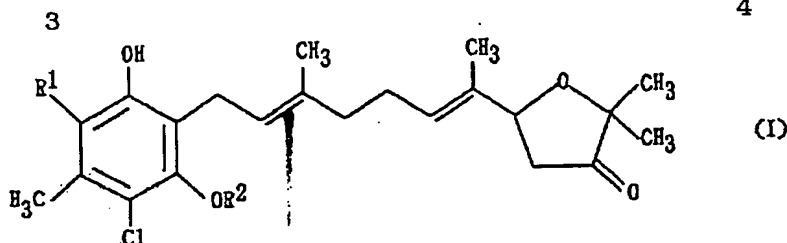


(式中R¹は-CHO又は-COOHを表し、R²は水素原子、-CH₂COOH又は式-CH₂COOR³ (式中、R³は炭素数1-6の低級アルキル基を表す)を表す。但し、R¹が-CHOでかつR²が水素原子又は-C

◆表す)である場合を除く)で示されるアスコフラノン誘導体及びその薬剤学的に許容し得る塩のうち少なくとも一種を有効成分として含有するグリケイション阻害剤。

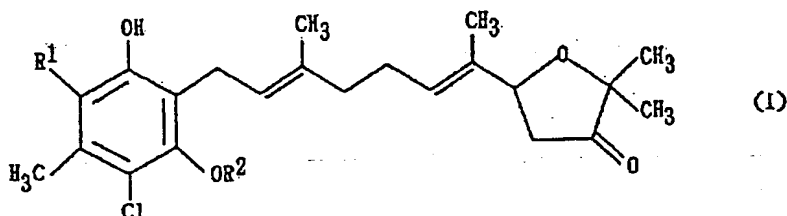
【請求項5】一般式 (I)

【化5】



(式中 R^1 は $-\text{CHO}$ 又は $-\text{COOH}$ を表し、 R^2 は水素原子、 $-\text{CH}_2\text{COOH}$ 又は式 $-\text{CH}_2\text{COOR}^3$ (式中、 R^3 は炭素数1-6の低級アルキル基を表す)を表す。但し、 R^1 が $-\text{CHO}$ でかつ R^2 が水素原子又は $-\text{CH}_2\text{COOR}^3$ (R^3 は炭素数1-6の低級アルキル基を表す)である場合を除く)で示されるアスコフラノン誘導体又はその薬剤学的に許容し得る塩のうち少なくとも一種を有効成分として含有する抗酸化剤。

【発明の詳細な説明】



(式中 R^1 は $-\text{CHO}$ 又は $-\text{COOH}$ を表し、 R^2 は水素原子、 $-\text{CH}_2\text{COOH}$ 又は式 $-\text{CH}_2\text{COOR}^3$ (式中、 R^3 は炭素数1-6の低級アルキル基を表す)を表す。但し、 R^1 が $-\text{CHO}$ でかつ R^2 が水素原子又は $-\text{CH}_2\text{COOR}^3$ (R^3 は炭素数1-6の低級アルキル基を表す)である場合を除く)。

【0003】

【従来の技術】上記一般式(I)の R^1 が CHO 、 R^2 が H で示されるアスコフラノンは本発明者によって糸状菌 *Ascochyta visiae* Libert の菌体中より単離されたものであり(特開昭48-91278号公報参照)、その抗腫瘍活性はすでに報告されている(特公昭63-61929号公報参照)。

【0004】英国特許第1,498,334号明細書には、上記一般式における R^1 が $-\text{CHO}$ で R^2 がアシル基であるアスコフラノン誘導体が記載されており、当該誘導体が血圧降下作用を有することが開示されている。

【0005】特公平4-28705号公報には、上記一般式における R^1 が水素原子で R^2 が OH であるアスコフラノン誘導体等が記載されており、アスコフラノンを合成するための中間体として有用であることが教示されている。

【0006】

【発明が解決しようとする課題】本発明者はアスコフラノンの薬剤としての新たな有用性について鋭意研究を重ねた結果、アスコフラノン並びに一部のアスコフラノン誘導体が優れた血糖低下作用を有することを報告した(特願平5-18904号)。しかし、これらの化合物は、(1)水溶性に乏しく、経口的に投与すると血中濃度※50

*【0001】

【産業上の利用分野】本発明は下記一般式(I)で示される新規なアスコフラノン誘導体又はその薬剤学的に許容し得る塩並びにこれらの化合物のうち少なくとも一種を有効成分とする血中脂質低下剤、血糖低下剤、グリケイション阻害剤及び抗酸化剤に関する。

【0002】

【化6】

※度は上昇しにくい、(2)経口的に投与すると腸管内においてエステラーゼによって、容易にエステル結合が分解する、(3)通常の酸化剤に対して非常に不安定で有機化学的方法では1位のアルデヒド基を相当するカルボン酸に酸化することは困難である。

【0007】

【課題を解決するための手段】 R^2 が $-\text{CH}_2\text{COOR}^3$ で示される化合物をあらかじめエステラーゼ処理するかアルカリ処理することによって、容易に $-\text{CH}_2\text{COOH}$ の化合物が得られ、血糖低下、脂質低下、メイラード反応阻害作用に既存の物質に比べて、優れた効果が認められた。また、アスコフラノン並びにその誘導体(1)動物肝臓の摩砕物とともに一定時間、攪拌するか(2)酸化細菌として知られる一群の細菌、例えば、*Pseudomonas*属あるいは*Acetobacter*属によって酸化させれば、1位の CHO 基を COOH 基に転換することができる。このようにして得られた1位が COOH に酸化された化合物は水溶性が増加し、経口投与によって血中濃度は上昇し、バイオアベイラビリティ(bioavailability)が増加する。また、経口投与による急性毒性は1/2-1/4に低下した。

【0008】以上の方法によって得られた本発明の目的化合物は、いずれも新規化合物で優れた血糖低下、脂質低下、メイラード反応阻害、抗酸化作用を有し、医薬品として有用であることを見出した。

【0009】本発明はこのような知見に基づいてなされたもので、上記一般式(I)で示されるアスコフラノン誘導体及びその薬剤学的に許容される塩、並びにこのよ

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うな化合物のうち、少なくとも一種を有効成分として含有する血中脂質低下剤、血糖低下剤、グリケイション阻害剤及び抗酸化剤を提供する。

【0010】本発明の抗酸化剤は、腎疾患、消化器系疾患、循環器系疾患、皮膚疾患等の各種疾患において、酸化作用を防止する目的で広く適用することができる。

【0011】本発明に係る各医薬の用量は病態の種類、症状によって異なるが例えば、注射の場合は成人一日一人当たり10-200mg、経口投与の場合には60-5000mg、座薬の場合には100-2000mgで目的を達成することができる。本発明の化合物は単独で用いてもよいが、通常はアルカリで中和して水に溶解したり、懸濁液、賦形剤又はその他の補助剤として混合し*

化合物番号	R ¹	R ²	R ³
1	CHO	CH ₂ COOH	-
2	COOH	CH ₂ COOR ³	CH ₃
3	COOH	CH ₂ COOH	-
4	COOH	II	-

なお、上記の化合物以外に、R¹がCOOHでR²がCH₂COOR³の化合物としては、R³がC₂H₅、n-C₃H₇、i-C₃H₇、n-C₄H₉、s-C₄H₉、i-C₄H₉、t-C₄H₉、n-C₅H₁₁、i-C₅H₁₁、n-C₆H₁₃などの化合物を挙げることができる。

【0014】以下に本発明を実施例に基づいて詳細に説明するが、これらは本発明を何ら限定するものではない。

【0015】

【実施例1】アスコフラン10.08gをジメチルホルムアミド120mlに溶解し、これに60%水素化ナトリウム0.8gを少しずつ加えた。得られたナトリウム塩の溶液にブrom酢酸メチルエステル6gを加えた。室温に一夜放置した後、さらに60%水素化ナトリウム0.08g及びブrom酢酸メチルエステル0.6gを加えた。一夜放置した後、減圧濃縮した。残った油状物に1%塩酸400ml及びクロロホルム400mlを加えて分液ロートにて攪拌し、クロロホルム層を分取し濃縮乾固した。残った油状物にメタノールを加えて一夜放置すると4-O-methoxycarbonylmethyl ascofuranone (AF-3913)の結晶7.6gが析出した。

【0016】得られた4-O-methoxycarbonylmethyl ascofuranone 2gを80mlのメタノールに溶解して、1N水酸化ナトリウム30mlと水130ml混合した溶液に加え、室温にて反応させた。30分後エチルエーテルにて洗滌後、冷却しながら2N-塩酸にてpH3とし酢酸エチルにて3回抽出した。酢酸エチル層を飽和食塩水にて洗滌後、無水硫酸ナトリウムにて脱水後、濃縮乾固すると4-O-carboxymethyl ascofuranone (化合物番号1の化合物、AF-M)の結晶

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*て非経口投与及び経口投与に適する剤形として製剤することが好ましい。好ましい製剤としては、例えば、注射剤、粉剤、顆粒剤、錠剤、糖衣錠、丸錠、カプセル剤、座剤等が上げられる。これらの製剤は常法により、例えば賦形剤または補助剤として、乳糖、蔗糖、種々の澱粉、ぶどう糖、セルロース、メチルセルロース、カルボキシメチルセルロース、ステアリン酸マグネシウム、ラウリル硫酸、タルク、植物油、レシチンならびにこれらの2種以上の混合物等を用いて製造される。

【0012】一般式(I)で示される本発明の化合物のうち、代表的な化合物を以下に示す。

【0013】

※1.4gが得られた。

【0017】化合物番号1の化合物(AF-M)のNMR分析データを以下に示す。

【0018】NMR δ (CDCl₃): 12.51 (1H, s), 10.27 (1H, s), 5.51 (1H, t, J=7.0Hz), 5.08 (1H, t, J=6.2Hz), 4.65 (1H, d, J=15.8Hz), 4.64 (1H, m), 4.59 (1H, d, J=15.8Hz), 3.46 (2H, d, J=7.0Hz), 2.64 (3H, s), 2.49 (1H, dd, J=18.3Hz, 6.6Hz), 2.44 (1H, d, J=18.3Hz, 10.1Hz), 2.18 (2H, m), 2.05 (2H, t, J=7.0), 1.77 (3H, s), 1.62 (3H, s), 1.31 (3H, s), 1.25 (3H, s)

【0019】

【実施例2】2%ぶどう糖、0.1%酵母エキス、0.2%ペプトン、0.1%塩化アンモニウム、0.05%リン酸-1-カリ、0.02%硫酸マグネシウム、0.5%炭酸カルシウムからなる培養培地10lに2%ぶどう糖添加肉汁培地に28℃で一晩培養したPseudomonas ovalis 200mlを接種した。培養は温度28℃で通気攪拌培養した。15時間後にTween80に溶解したascofuranone 20gを添加し、さらに培養を継続した。2日後に培養を終了させ、遠心分離機にて分離して得られた培養濾液に硫酸を加えてpH3に調節した。次に酢酸エチル5lを加えて攪拌抽出した。酢酸エチル層を分離し、無水硫酸ナトリウムにて脱水した後、減圧濃縮した。得られた油状物をベンゼン・シリカゲルカラムクロマトグラフィーを行い、ベンゼン-アセトン(3:1)混液で溶出した画分を濃縮乾固することによって化合物番号4の物質を得

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た。

【0020】化合物番号4の化合物のNMR分析データを以下に示す。

【0021】NMR δ (CDCl₃) : 9.66 (1H, s), 5.43 (1H, brt), 5.13 (1H, brt), 4.46 (1H, t J=8.3Hz), 3.30 (2H, d J=6.4Hz), 2.53 (3H, s), 2.36 (2H, m), 1.90-2.15 (4H, m), 1.74 (3H, s), 1.54 (3H, s), 1.11 (3H, s), 1.14 (3H, s)

【0022】

【実施例3】1群8匹のddYマウスにアスコフラノン (AF)、4-O-メトキシカルボニルメチル アスコフラノン (AF-3913)、4-O-カルボキシメチルアスコフラノン (AF-M) を8mg/kg 7日間経口投与し、血中コレステロール、血中中性脂肪に与える効果を検討した。結果は表1に示した。

【0023】

【表1】

マウスにおける血中コレステロール、中性脂肪値

	n	コレステロール (mg/dl)	中性脂肪 (mg/dl)
対照群	8	116±10	199±13
AF	8	113±9 ns	159±10 -20% *
AF-3913	8	98±8 -16% *	146±12 -27% **
AF-M	8	95±8 -18% **	138±11 -31% **

平均±標準誤差 * p<0.05 ** p<0.01

AF-M投与群の血中コレステロール、中性脂肪値は対照群に比べて有意に低下したが、その低下率は他の2検体に比べて大きかった。

【0024】

【実施例4】25mg/mlの牛血清アルブミンに200mMのグルコースを加え、アスコフラノン (AF) 並びにその誘導体 (AF-3913, AF-M) を40, 200, 1000, 5000 μ g/mlの割合で添加し、37℃、14日間培養し、生成したケトアミンの加水分解産物フロシンを高速液体クロマトグラフィーで測定した。結果は表2に示した。

【0025】

【表2】

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フロシン生成に対する効果

	阻害率 (%)			
μ M	5000	1000	200	40
AF	22	20	5	0
AF-3913	23	19	6	0
AF-M	35	27	10	0

AF-M添加は他の2検体の場合に比較して、フロシン生成阻害率は高値を示した。

【0026】

【実施例5】10mMのリボース、アルギニン、リジンを含む反応液にアスコフラノン (AF) 並びにその誘導体 (AF-3913, AF-M) を1000, 500, 100, 20 μ M添加して37℃8日間培養し、生成したペントシジンを高速液体クロマトグラフィーで測定した。結果は表3に示した。

【0027】

【表3】

ペントシジン生成に対する効果

	阻害率 (%)			
μ M	1000	500	100	20
AF	100	85	60	25
AF-3913	100	88	65	28
AF-M	100	95	77	38

AF-M添加は他の2検体の場合に比較して、ペントシジン生成阻害率は高値を示した。

【0028】

【実施例6】ラット肝臓ミトコンドリアにアスコルビン酸100 μ M、塩化第二鉄25 μ Mを加え、37℃、1時間、培養し、生成する過酸化脂質に対するAF-3913とAF-Mの効果を検討した。結果は表4に示した。

【0029】

【表4】

過酸化脂質生成に対する効果

	μ g/ml	過酸化脂質	ng/ml
対照		62.1±1.1	
AF-3913	1000	9.6±0.6	-80.0% **
	100	47.2±1.8	-9.2% *
	10	55.1±2.4	ns
AF-M	1000	13.4±1.1	-74.8% **
	100	36.7±2.3	-30.9% **
	10	45.8±0.8	-13.8% *

平均±標準誤差 * p<0.05 ** p<0.01
両化合物ともに過酸化脂質の生成を抑制したが、AF-MはAF-3913に比較してより低濃度で有効であった。

50 【0030】

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【発明の効果】上記式(I)で示される新規なアスコフ
ラノン誘導体又はその薬剤学的に許容し得る塩は、優れた
血中脂質低下作用、血糖低下作用、グリケイション阻

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害作用及び抗酸化作用を示すので、糖尿病、動脈硬化症
等の予防、治療薬として極めて有用である。

フロントページの続き

(51)Int.Cl.⁶
A61K 31/34

識別記号
AED

庁内整理番号

FI

技術表示箇所